

What is claimed is:

1. A method of providing sperm comprising:
  - a) cooling a sample which includes sperm to a first temperature sufficient to protect sperm from glycerol toxicity, at a rate sufficiently slow that the metabolic rate of sperm is decreased, to provide a cooled sperm sample;
  - b) adding a solution comprising glycerol to the cooled sperm sample; and
  - c) freezing said cooled sperm sample to second temperature for a sufficient period of time to equilibrate glycerol and sperm to thereby provide a frozen sperm sample, such that the sperm is preserved.
2. The method of claim 1, further comprising: providing a sperm sample to be cooled in a cryoprotectant buffer lacking glycerol.
3. The method of claim 1, wherein the cryoprotectant buffer comprises about 10% to about 30% egg yolk.
4. The method of claim 1, wherein the concentration of glycerol in the sample after the addition of the glycerol solution is about 5% to about 10% glycerol.
5. The method of claim 1, wherein the sperm sample is obtained from a mammal.
6. The method of claim 1, wherein the first temperature is between about 0°C to about 10°C.
7. The method of claim 1, wherein the sperm sample is maintained at the first temperature for between about 4 hours to about 21 hours.
8. The method of claim 1, wherein the sperm is cooled at a rate of between about 0.2°C to about 0.5°C per minute to the first temperature.

9. The method of claim 1, wherein the sperm is cooled to the first temperature over the course of about 1.5 to about 4 hours.

10. The method of claim 1, wherein the second temperature is between about -40°C to about -100°C.

11. The method of claim 1, wherein the sample is maintained at the second temperature for between about 7 minutes to about 20 minutes.

12. The method of claim 1, wherein the method further comprises storing the frozen sperm sample at a third temperature of about -190°C to about -200°C.

13. A method of preserving sperm comprising:

- a) combining sperm with a first cryoprotectant buffer;
- b) cooling said sperm to a first temperature between about 2°C to about 10°C at a rate sufficiently slow that the metabolic rate of sperm is decreased to produce cooled sperm;
- c) freezing the cooled sperm at a second temperature between about -60°C to about -90°C; and
- d) storing the frozen sperm in liquid nitrogen.

14. The method of claim 13, wherein the first cryoprotectant buffer comprises about 5% to about 10% glycerol.

15. The method of claim 13, wherein the first cryoprotectant buffer lacks glycerol.

16. The method of claim 13, wherein the sperm sample is obtained from a mammal.

17. The method of claim 13, wherein the sperm sample is maintained at the first temperature for between about 4 hours to about 21 hours.

18. The method of claim 13, wherein the sperm is cooled at a rate of between about 0.2°C to about 0.5°C per minute to the first temperature.

19. The method of claim 13, wherein the sperm is cooled to the first temperature over the course of about 1.5 hours to about 4 hours.

20. The method of claim 15, wherein a second cryoprotectant buffer is added to the sample after the sperm is cooled to the first temperature, and before the sperm is further cooled to the second temperature.

21. The method of claim 20 wherein the second cryoprotectant buffer comprises about 5% to about 10% glycerol.

22. The method of claim 13, wherein the second temperature is about -80°.

23. The method of claim 13, wherein the sample is maintained at the second temperature for between about 7 minutes to about 20 minutes.

24. A method of providing sperm comprising:

- a) providing a sample comprising sperm;
- b) isolating sperm from the sample;
- c) combining said isolated sperm with a first cryoprotectant buffer which lacks glycerol;
- d) cooling said sperm to a first temperature of about 2°C to about 8°C at a rate of about 0.2 °C to 0.5°C per minute to produce cooled sperm;
- e) adding a second cryoprotectant buffer which contains glycerol;
- f) maintaining the cooled sperm at the first temperature for a duration of about 4 hours to about 21 hours;
- g) freezing the cooled sperm at a second temperature of about -60°C to about -90°C for a time of between about 10 minutes to about 15 minutes;

- 91 h) storing the frozen sperm at a temperature of between about -180° to about -220°C  
92 for a desired time period; and  
93 i) thawing said sperm, to thereby provide sperm.

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95 25. The method of claim 24, wherein the sperm are thawed for about 90 seconds in a water  
96 bath at about 37°C

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98 26. A method of making an animal, comprising fertilizing an oocyte with sperm preserved by  
99 the method of claim 1, claim 13, or claim 24.

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FOOTNOTES